Mucosal Monosaccharide Transporter Expression in Newborns With Jejunoileal Atresia and Along the Adult Intestine

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Abstract: OBJECTIVES: In newborn rodents, intestinal maturation involves delayed fructose transporter GLUT5 expression until weaning. In jejunoileal atresia (JIA), distal intestinal segments lack exposure to amniotic fluid-containing carbohydrates. We assessed in human newborns, the impact of intestinal maturation and obstruction on mucosal monosaccharide transporter expression. METHODS: Samples were obtained from 10 newborns operated for small intestinal atresia and from 17 adults undergoing gastroduodenoscopy and/or ileocolonoscopy. mRNA expression of the transporters SGLT1, GLUT1, GLUT2, GLUT5, and GLUT7 was measured in neonate samples proximal and distal of the atresia as well as in adult duodenum, ileum, and colon. Protein expression and localization was assessed using immunofluorescence. RESULTS: Although mRNA expression of monosaccharide transporters did not significantly differ between newborn and adult samples, luminal fructose transporter GLUT5 protein was absent in 0- to 4-day-old neonates, but expressed in adults. The mRNA expression of the 5 tested monosaccharide transporters was unchanged distal from the JIA relative to proximal. Similarly, luminal sodium-dependent glucose transporter SGLT1 and basolateral GLUT2 were expressed proximal and distal to JIA as visualized by immunofluorescence staining. With the exception of glucose transporter GLUT1 that showed highest expression levels in colon, all investigated hexose transporters showed strongest expression in duodenum, lower levels in ileum and lowest in colon. CONCLUSIONS: Human newborns lack small intestinal fructose transporter GLUT5 protein expression and small intestinal atresia does not affect the expression of hexose transporters.

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ABSTRACT

Objectives: In newborn rodents, intestinal maturation involves delayed fructose transporter GLUT5 expression until weaning. In jejunoileal atresia (JIA), distal intestinal segments lack exposure to amniotic fluid-containing carbohydrates. We assessed in human newborns, the impact of intestinal maturation and obstruction on mucosal monosaccharide transporter expression.

Methods: Samples were obtained from 10 newborns operated for small intestinal atresia and from 17 adults undergoing gastroduodenoscopy and/or ileocolonoscopy. mRNA expression of the transporters SGLT1, GLUT1, GLUT2, GLUT5, and GLUT7 was measured in neonate samples proximal and distal of the atresia as well as in adult duodenum, ileum, and colon. Protein expression and localization was assessed using immunofluorescence.

Results: Although mRNA expression of monosaccharide transporters did not significantly differ between newborn and adult samples, luminal fructose transporter GLUT5 protein was absent in 0- to 4-day-old neonates, but expressed in adults. The mRNA expression of the 5 tested monosaccharide transporters was unchanged distal from the JIA relative to proximal. Similarly, luminal sodium-dependent glucose transporter SGLT1 and basolateral GLUT2 were expressed proximal and distal to JIA as visualized by immunofluorescence staining. With the exception of glucose transporter GLUT1 that showed highest expression levels in colon, all investigated hexose transporters showed strongest expression in duodenum, lower levels in ileum and lowest in colon.

Conclusions: Human newborns lack small intestinal fructose transporter GLUT5 protein expression and small intestinal atresia does not affect the expression of hexose transporters.

Key Words: GLUT2, GLUT5, human newborns, intestine, monosaccharide absorption, SGLT1

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What Is Known

- Small intestinal atresia prevents intestinal exposure to amniotic fluid.
- The expression of hexose transporters in the human newborn gut is poorly studied.

What Is New

- Fructose transporter GLUT5 mRNA but not protein is expressed in the newborn small intestine.
- Small intestinal atresia does not seem to affect hexose transporter expression.
- Human newborn intestine is not equipped for fructose absorption in the first days of life.

Intestinal monosaccharide absorption involves luminal uptake and basolateral efflux mediated by enterocyte transporter families SGLT and GLUT (1,2). The mostly studied intestinal monosaccharide transporters belonging to these 2 families are SGLT1 (SLC5A1), GLUT2 (SLC2A2), and GLUT5 (SLC2A5) (3,4). SGLT1 is a brush border membrane (BBM) glucose and galactose symporter (5), its defect resulting in ose-galactose malabsorption (5,6). The uniporter GLUT2 is expressed in the basolateral membrane, transporting glucose, galactose, and fructose. GLUT5 exclusively transports fructose across the BB (7). The transporter GLUT7 was more recently described, and is highly expressed in the small intestine. Its substrate is not known yet (8). Glucose transporter GLUT1 (SLC2A1), although normally hardly present in enterocytes, is highly expressed in cells undergoing oncogenic transformation, reflecting the increased cellular glucose metabolism (9).

Intestinal glucose uptake can be detected in human fetuses at 11 weeks of gestation and increases with further development (10). In newborn rodents, GLUT2 and SGLT1 are already expressed at birth, whereas GLUT5 expression is delayed until weaning is completed (11–13). Expression of intestinal monosaccharide transporters in human newborns has yet to be completely studied. The capacity of the small intestine to absorb monosaccharides depends, among other factors, on the abundance of monosaccharide transporters (14). Lack of functional small intestine that is commonly seen after major intestinal resection (eg, in newborns with necrotizing enterocolitis), leads to short bowel syndrome (15). Knowledge about the longitudinal expression of nutrient transporters may affect clinical decision-making when parts of the small intestine need to be resected. A first aim of the present study was hence to

assess the expression of monosaccharide transporters in their first days after birth.

Amniotic fluid (AF), containing many nutrients, is considered a regulatory fuel during intrauterine growth, and is swallowed by the fetus (13,16,17). This constant swallowing is blocked in neonates with jejuno-ileal atresia (JIA), a complete intestinal obstruction resulting from disrupted mesenteric blood supply (18). Experimental disruption of amniotic fluid passage in animals led to altered intestinal mucosa development (19) and decreased somatic growth (20). Similarly, newborns with bowel resection for small intestinal atresia may show malabsorption and delayed growth correlating with the remaining length of functional small bowel (21). Whether this is in part attributed to a reduced nutrient transport capacity of the remaining bowel distal to the atresia is not known. A second aim of the present study was hence to assess the effect of JIA on small intestinal monosaccharide transporter expression.

MATERIALS AND METHODS

Study Population and Sample Collection

Newborns

Newborns with jejunal or ileal atresia were included in the present study. In patients with multiple atresias, only tissue immediately adjacent to the most proximal atresia was used for further analysis. Patients were treated at the University Children's Hospitals in Basel and in Zurich. Ten (6 male and 4 female infants) newborns were included. Thereof, 5 patients had complete jejunal and 4 patients had complete ileal atresia. One patient suffered from ileal stenosis and was hence excluded from analyses proximal versus distal to atresia, but was included in analyses of newborns versus adults (Table 1).

Age at surgery ranged from 0 to 4 days (median: 2 days), and patients' gestational age ranged from 34 + 3/7 to 39 weeks with 4 of 10 newborns being born preterm. Newborn's birth weight ranged from 2050 to 3900 g (median: 3040 g). After tissue removal in the operating room, tissue samples were either cut (for later cryosections and immunofluorescence) or the mucosa was scraped off the submucosa using the backside of a scalpel (for later RNA extraction). Specimens where transferred into 1.5 mL microcentrifuge tubes (Eppendorf tubes® 3810X, Eppendorf AG, Hamburg, Germany), immersed in liquid nitrogen, and stored at −80°C until further processing.

Adults

Seventeen adult patients (9 men and 8 women) were included in the present study. Median patient's age was 62 years (range: 41–77 years). Patients underwent gastroduodenoscopy, combined gastroduodenoscopy and ileocolonoscopy, or ileocolonoscopy as part of a routine medical checkup at the University of Zurich, Switzerland, Department of Gastroenterology. Patients younger than 18 years or older than 80 years were excluded, as well as patients with pathologies of the gastrointestinal tract. Mucosal biopsies were obtained from the descending/horizontal part of the duodenum (13 patients), the terminal ileum (5 patients), and from the ascending colon (9 patients). After removal, tissue samples were transferred to 1.5 mL tubes, immediately frozen in liquid nitrogen and stored at −80°C.

TABLE 1. Clinical characteristics of newborn patients

Clinical characteristics	Data
Sex, n (%)	
Female	4 (40)
Male	6 (60)
Age at surgery, mean [days] (range)	2 (0–4)
Gestational age, mean [weeks] (range)	37.3 (34.4–41.3)
Birth weight, mean [g] (range)	2874 (2430–3900)
Type of defect, n (%)	
Atresia	9 (90)
Stenosis	1 (10)
Location of defect, n (%)	
Ileum	5 (50)
Jejunum	5 (50)

Cryosections and Immunofluorescence

Fresh frozen tissue was cut into 5 μm thick cryosections as described elsewhere (22). Briefly, samples were embedded in optical cutting temperature (OCT) compound (Medite Medizinaltechnik AG, Switzerland), cut with a cryotome (Leica CM 1850 Cryostat, Switzerland), transferred onto polysine slides (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and stored at −20°C until further processing. Before fixation, slides were allowed to thaw for 5 minutes in a wet chamber. Thereafter, slides were fixed for either 90 seconds in methanol at −20°C (GLUT2) or for 30 minutes in 3% paraformaldehyde (PFA; GLUT5 and SGLT1) and then washed in phosphate-buffered saline (PBS). PFA-fixated slides underwent heat-induced epitope retrieval in sodium citrate pH 6 at 98°C in a microwave pressure cooker (HistosPRO [SW 2.0.0] Rapid Microwave Histoprocessor, Milestone Srl, Italy) for 10 minutes. In order to block unspecific binding, specimens were incubated for 30 minutes with 2% bovine serum albumin (BSA) in PBS containing 0.04% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA) before incubation with primary antibodies. The primary antibodies used were polyclonal rabbit anti-GLUT2, polyclonal rabbit anti-GLUT5 and polyclonal rabbit anti-SGLT1 (catalogue numbers HPA028997, HPA005449, and HPA055106, all Sigma-Aldrich, St. Louis, Missouri, USA). Antibodies were diluted 1 : 300 (GLUT2), 1 : 200 (GLUT5), and 1 : 500 (SGLT1) and incubated on specimen for 1 hour at room temperature. After incubation, slides were washed twice in hypertonic PBS, additionally containing 18 g NaCl/L, and once in normal PBS. Thereafter, specimens were incubated with an Alexa Fluor 488-conjugated polyclonal donkey anti-rabbit IgG antibody (catalogue #ab150061, Abcam, Cambridge, United Kingdom) diluted 1:500 and 4',6-Diamidino-2-Phenylindole (DAPI; Invitrogen, Carlsbad, California, USA) for 1 hour at room temperature. Slides were washed twice in hypertonic and once in conventional PBS and mounted with coverslips using an aqueous glycergel mounting medium (Agilent Technologies, Santa Clara, USA).

For analysis, we used a Nikon Eclipse TE300 epifluorescence microscope equipped with a DS-5M Standard charge-coupled device camera. Images were acquired via the NISElements software (all from Nikon Instruments, Inc., Tokyo, Japan) and were merged in Adobe Photoshop CS5. Scale bars were added using Fiji/ImageJ (SciJava). All image acquisition and processing steps were performed applying standardized conditions for each antibody, thereby ensuring comparability.

RNA Extraction and Real-time Reverse Transcriptase Polymerase Chain Reaction

First, frozen biopsies (adults) and frozen scraped mucosa (newborns) were homogenized with the use of MagNa Lyser Green Beads (Roche, Basel, Switzerland). For RNA extraction, a RNeasy Mini Kit (Qiagen, Venlo, Netherlands) was used following the manufacturer's instructions. RNA quantity and quality were assessed with a ND-1000 NanoDrop UV-spectrophotometer (NanoDrop Technologies, Wimington, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), respectively.

After reverse transcription of 8 ng/μL RNA with the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA), we performed quantitative PCR (qPCR) for GLUT1, GLUT2, GLUT5, GLUT7, and SGLT1, taking 8 ng of cDNA as template. All qPCR reactions were carried out in duplicates using the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA) in a Prism 7700 cycler (Applied Biosystems, Foster City, California, USA). Reactions were carried out as duplex, using target gene primers and probes (Microsynth, Balgach, Switzerland) and eukaryotic 18S rRNA as endogenous control (Applied Biosystems, Foster City, California, USA) simultaneously. Primer and probe sequences are listed in Supplemental Digital Content 1 (*http://links.lww.com/MPG/B671*). Expression levels of target mRNA relative to the housekeeping 18S rRNA were calculated as described previously (22).

Ethics

This study was approved by the local ethics committee (biopsies from adults: *EK-1744;* biopsies from newborns: *KEK-ZH-Nr. 2010-0387/EKNZ 2015-182*). Written informed consent was obtained from all patients (adult samples), respectively, from patients' caregivers (newborn samples).

Statistics

For graphic representation (real-time PCR data) and statistical analysis, the statistical software Graphpad Prism 5 (GraphPad Software, San Diego, CA) was used. P values of ≤ 0.05 were considered statistically significant. Represented bars show mean and standard error of the mean (SEM). Statistical tests included 1-way analysis of variance (ANOVA) with Bonferroni's comparison of all pairs of columns (for nonrepeated parametric data), 2-tailed unpaired *t*-tests (for unpaired parametric data) and 2-tailed paired *t*-tests (for paired parametric data).

RESULTS

Expression of Monosaccharide Transporter mRNAs in Intestine of Human Adults and Newborns

Mucosal biopsies from the duodenum, terminal ileum and ascending colon were taken from 17 adult patients undergoing gastroduodenoscopy and/or ileocolonoscopy, and the monosaccharide transporter mRNA expression relative to 18S rRNA was measured using real-time reverse transcriptase polymerase chain reaction (rt-PCR). The mRNA levels of monosaccharide transporters GLUT2, GLUT5, GLUT7, and SGLT1 were highest in the duodenum, significantly lower (2–10-fold) in ileum and even lower in distal colon. In contrast, GLUT1 showed a significantly higher mRNA level in colon compared with duodenum and ileum ($P \leq 0.001$), which displayed similar lower levels (Fig. 1A–E).

The mRNA expression levels of monosaccharide transporters (relative to 18S RNA) measured in terminal ileum mucosal biopsies of adults were compared with the levels measured in surgical specimens (mean value of proximal and distal samples) from jejunum and ileum of human newborns. Interestingly, mRNA levels were not significantly different for any of the tested monosaccharide transporters, although there was a nonsignificant trend to higher values in newborns for GLUT1 ($P = 0.102$), GLUT2 ($P = 0.106$), and GLUT7 ($P = 0.057$) (Fig. 1F).

Surgical resection-margins proximal and distal to small intestinal atresia were processed and monosaccharide transporter mRNA levels measured using real-time rt-PCR. Interestingly, there was no difference between small intestinal segments proximal and distal to JIA (Fig. 1G).

Intestinal Monosaccharide Transporter Protein Expression in Intestine of Human Adults and Newborns

Immunofluorescence staining of intestinal biopsies from duodenum, terminal ileum, and ascending colon of adults was performed. GLUT2 staining was seen in the basolateral membrane in duodenum (Fig. 2A, F, and K) and terminal ileum (Fig. 2B, G, and I). GLUT5 was detected at the luminal membrane of enterocytes in duodenum (Fig. 3A, F, and K) and terminal ileum (Fig. 3B, G, and L). Similarly to GLUT5, SGLT1 apical staining was seen in duodenum (Fig. 4A, F, and K) and ileum (Fig. 4B, G, and L). Colonic samples did not show any staining for GLUT2, GLUT5, or SGLT1, indicating a very low expression level or the absence of these proteins (Figs. 2–4C, H, and M).

Immunofluorescence staining on newborn small intestinal segments revealed a signal for GLUT2 at the basolateral membrane of enterocytes and for SGLT1 at the luminal brush-border membrane, both in the segments proximal (Figs. 2 and 4D, I, and N) and distal (Figs. 2 and 4E, J, and O) to JIA, with no differences in subcellular localization. Interestingly, GLUT5 was not detectable in newborn small intestine (Fig. 3D, E, I, J, N, and O) whereas in adult duodenum and ileum, a clear staining of the apical enterocyte membrane was visible (Fig. 3A, B, F, G, K, and L).

DISCUSSION

Developmental Regulation of Intestinal Monosaccharide Transport

In view of the lack of other possibilities to obtain intestinal tissue from human newborn, the resection of JIA represents a unique opportunity. The resected tissue from proximal to the atresia had rather normal conditions of development. In contrast, the mucosa from beyond the atresia has not been exposed during late fetal life to the bolus made of swallowed amniotic fluid because of the interruption of the intestinal continuity. Thus, differences in intestinal mucosa development potentially because of the lack of passage of bolus containing amniotic fluid may be investigated (see below). We, thus, first studied the normal development of mucosal monosaccharide transporters and compared our findings in newborns with results obtained in samples taken from the ileum of adults during routine endoscopy.

We observed at the mRNA level that expression of intestinal monosaccharide transporters GLUT1, GLUT2, GLUT5, SGLT1, and of GLUT7 relative to the ribosomal RNA 18S, did not significantly differ between human newborns and adults, with GLUT1/2, and 7 expression showing a trend to be higher in newborns. This is in contrast with rats and rabbits where GLUT5 mRNA expression is delayed during development until the suckling and weaning period is completed (11). Similarly, as in the present study, SGLT1 and GLUT2 are already expressed at birth in rodents (11). A similar developmental regulation of SGLTl, GLUT1, GLUT2, and GLUT5 mRNA expression has been observed in a previous study of human fetuses: SGLT1 mRNA was first detected at 17 weeks of gestational age and reached adult levels by 19 weeks. GLUT2 mRNA was first detected at 11 weeks (the earliest time studied) and levels increased during pregnancy. In contrast, GLUT5 mRNA was barely detectable in fetal small intestine and its protein was reported to be localized at the intercellular junctions of the developing villus (23). In the present study, GLUT5 protein expression was, however, not detected in neonates ages 0 to 4 days, suggesting a deferred mRNA

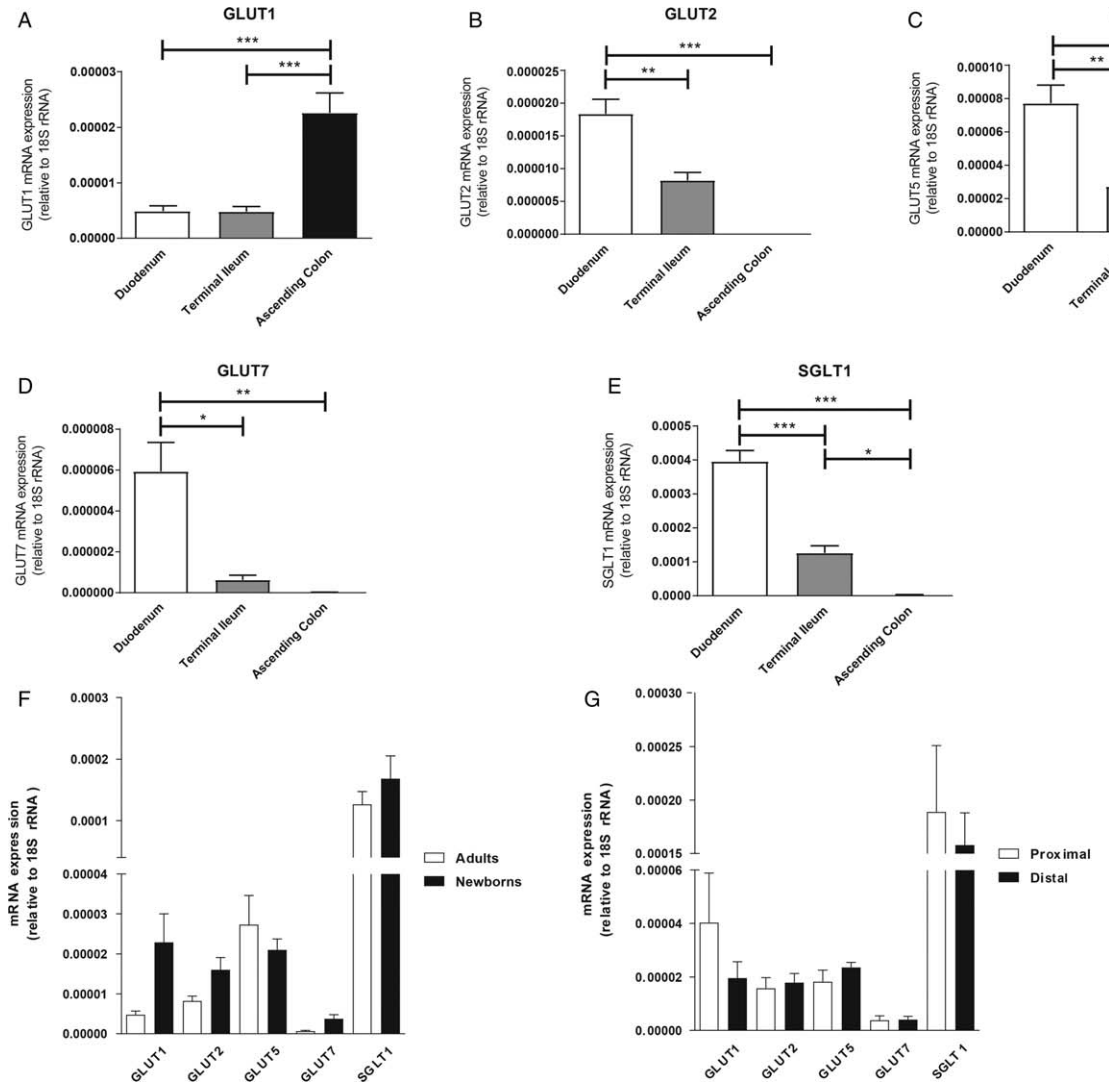

FIGURE 1. (A–E) mRNA expression of GLUT1, GLUT2, GLUT5, GLUT7, and SGLT1 in duodenum, terminal ileum, and ascending colon; nonrepeated ANOVA with Bonferroni comparison of all pairs of columns. (F and G) Monosaccharide transporter expression in adults versus newborns (F) and in newborns proximal versus distal of JIA (G); multiple unpaired 2-tailed t-tests. (A–E) Boxplots show the median and the upper (75th percentile) and lower (25th percentile) quartiles. The whiskers show maximal and minimal values. (F and G) Bars indicate mean ± SEM. Individual data points are given. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. ANOVA = analysis of variance; JIA = jejunoileal atresia; SEM = standard error of mean.

translation or protein stabilization. With GLUT5 not being expressed in the BBM, and in the absence of other still unknown BBM fructose transporters or efficient paracellular transport, newborns cannot efficiently absorb fructose in their first days of life. Fructose is not naturally present in maternal milk; however, it can be found in low concentrations in breast milk because of the mothers' diet (24). Symptoms of vomiting, regurgitation, and/or abdominal bloating, which may be seen in newborns after breast milk intake might hence be caused by temporary dietary fructose intolerance. The pathophysiology of fructose malabsorption is unknown, but is likely correlated to small intestinal GLUT5 expression (25). Its prevalence correlates with age, with infants more often being affected than older children (25,26). As dietary fructose restrictions ameliorate symptoms in children with fructose malabsorption, avoidance of high fructose diet in early lactating mothers might improve symptoms of breast milk intolerance in newborns.

On the contrary, GLUT2 protein was expressed in human newborns proximal and distal to small intestinal atresia. As in adults, GLUT2 protein localized exclusively to the basolateral enterocyte membrane. Also, SGLT1 showed a similar protein expression pattern in human newborn BBM of both proximal and distal to atresia, as in adult ileum.

In the present study, tissue samples from newborn jejunum or ileum were compared with adult terminal ileum specimens. Thus, differences in longitudinal transporter expression might hence have biased our results. Additionally, whereas specimens from adults were obtained from ileum during routine lower endoscopy, newborn samples were collected during surgery for small intestinal atresia and might hence be affected by the disease itself. Nevertheless, other indications to resect small intestine in newborns (such as, eg, necrotizing enterocolitis or volvulus) are likely to alter the intestinal mucosa more than small intestinal atresia. Another possible confounder in this study is the feeding state from the patients at

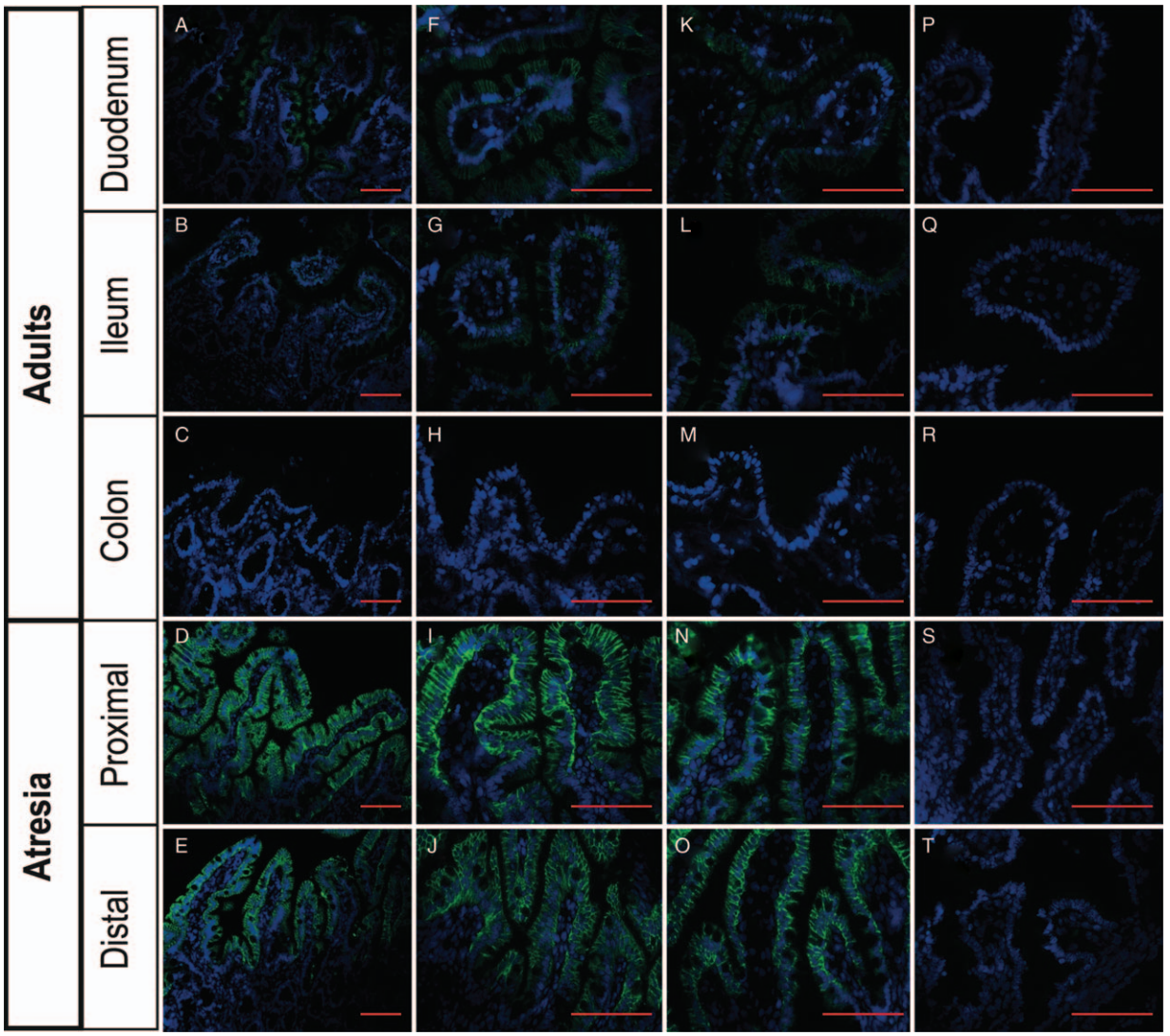

FIGURE 2. Immunofluorescence staining of GLUT2 (green). Cellular DNA (A–T; DAPI; blue) is shown to display the nuclei. Tissue specimens are derived from adult duodenum (A, F, and K), terminal ileum (B, G, and L), and ascending colon (C, H, and M) as well as from newborns proximal (D, I, and N) and distal (E, J, and O) to JIA. Negative controls without a primary antibody against GLUT2 are shown from adults in duodenum (P), terminal ileum (Q), and ascending colon (R), as well as from newborns proximal (S) and distal to atresia (T). Scale bars = 100 μm. JIA = jejunoileal atresia.

the sample collection time. Adult patients undergoing lower endoscopy were made ''nothing by mouth'' the night prior endoscopy, whereas in some newborns, an oral feeding attempt might have been undertaken before the intestinal obstruction became evident.

Intestinal Occlusion Does Not Affect Monosaccharide Transporter Expression

Jejunoileal atresia likely results from a vascular event during late pregnancy compromising mesenteric blood supply to a small bowel segment. This hypothesis is supported by animal models with JIA development following in utero ligation of mesenteric blood vessels (27). Furthermore, maternal exposure to vasoconstrictive agents during pregnancy increases the likelihood of JIA development (18). At term, the fetus swallows around 3/4 L of amniotic fluid per day. The amniotic fluid contains many biologically relevant compounds, such as carbohydrates, amino acids, proteins, peptides, lipids, and hormones that have been suggested to facilitate small intestinal and fetal growth (20,28). This important role of luminal amniotic fluid components has indeed been experimentally verified in animals in which proximal intestinal obstruction was shown to result in a decrease in intestinal villus height and somatic growth (20,29–31). In the presented study, small intestinal atresia surprisingly had no significant effect on the expression of GLUT1, GLUT2, GLUT5, GLUT7, or SGLT1 mRNA. Furthermore, qualitative analysis of GLUT2 and SGLT1 IF staining showed similar patterns proximal and distal to the intestinal atresia. These findings, however, confirm previous results showing unimpaired GLUT2 expression proximal and distal to JIA (32), and add information on the expression of monosaccharide transporters GLUT5 and SGLT1, which had not been studied previously.

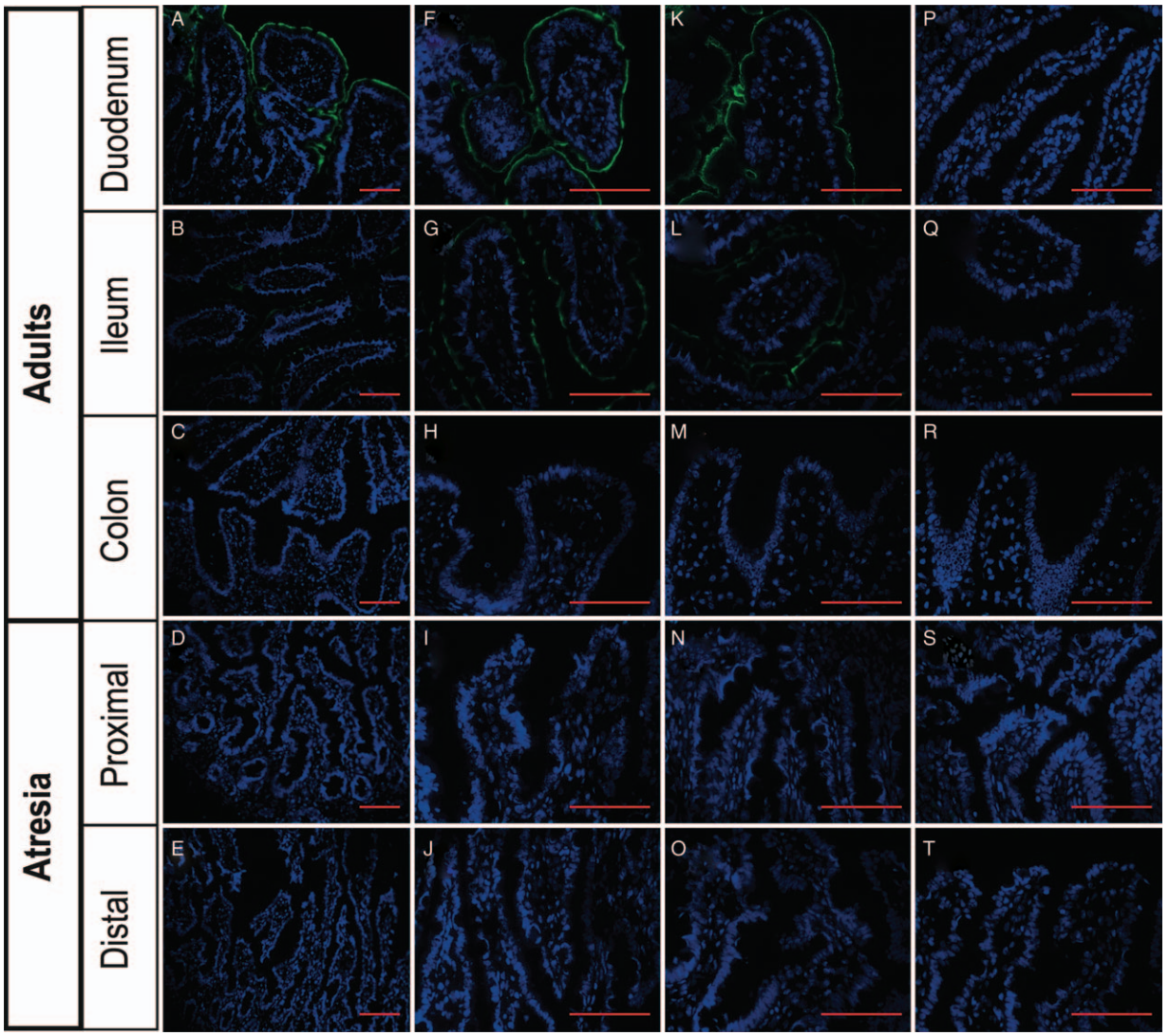

FIGURE 3. Immunofluorescence staining of GLUT5 (green). Nuclear DNA (A–T; DAPI; blue) is shown to display the nuclei. Tissue specimens are derived from adult duodenum (A, F, and K), terminal ileum (B, G, and L), and ascending colon (C, H, and M) as well as from newborns proximal (D, I, and N) and distal (E, J, and O) to JIA. Negative controls without a primary antibody against GLUT5 are shown from adults in duodenum (P), terminal ileum (Q), and ascending colon (R), as well as from newborns proximal (S) and distal to atresia (T). Scale bars = 100 μm. JIA = jejunoileal atresia.

Monosaccharide Transporter Expression Along the Adult Human Intestine

Information about the longitudinal distribution of monosaccharide transporters in the human intestine are sparse and incomplete in the literature (23). Unlike amino acid transporters, showing variable transporter expression alongside the digestive tract of humans (22) and rodents (33), the transporters GLUT2, GLUT5, GLUT7, and SGLT1 uniformly exhibited a decreasing mRNA expression pattern from proximal (duodenum) to distal (colon) in the present study. This data resembles the mRNA expression pattern previously observed in mice (34), rats (35), and also in humans as published in the public domain on website ''The Human Protein Atlas'', where GLUT2 and GLUT7 were expressed at the RNA and at the protein level in duodenum and small intestine, but not in colon. Similarly, GLUT5 and SGLT1 showed much higher RNA and protein expression levels in duodenum and small intestine than in colon (*https://www.proteinatlas.org*).

Interestingly, the small intestine lacks the expression of high-capacity low-affinity transporter SGLT2 (SLC5A2) to complement the expression of SGLT1, a high-affinity low-capacity transporter, as it is observed in the kidney. In kidney proximal tubule, SGLT2 is expressed in the early convoluted segments S1 and S2 where the bulk of glucose reabsorption takes place. SGLT1 is, therefore, primarily expressed in the more distal proximal tube (straight segment S3) and enables the uptake of remaining luminal glucose (36,37). Contrary to kidneys, the low-affinity transporter SGLT2 is absent in human intestine (38).

GLUT1 is highly expressed in different cell types, in many tumors (39–43), and in various fetal tissues including lungs and intestine, likely reflecting increased glucose metabolism because of cell proliferation and maturation (23,44). Our results showed higher GLUT1 mRNA expression in the large intestine when compared

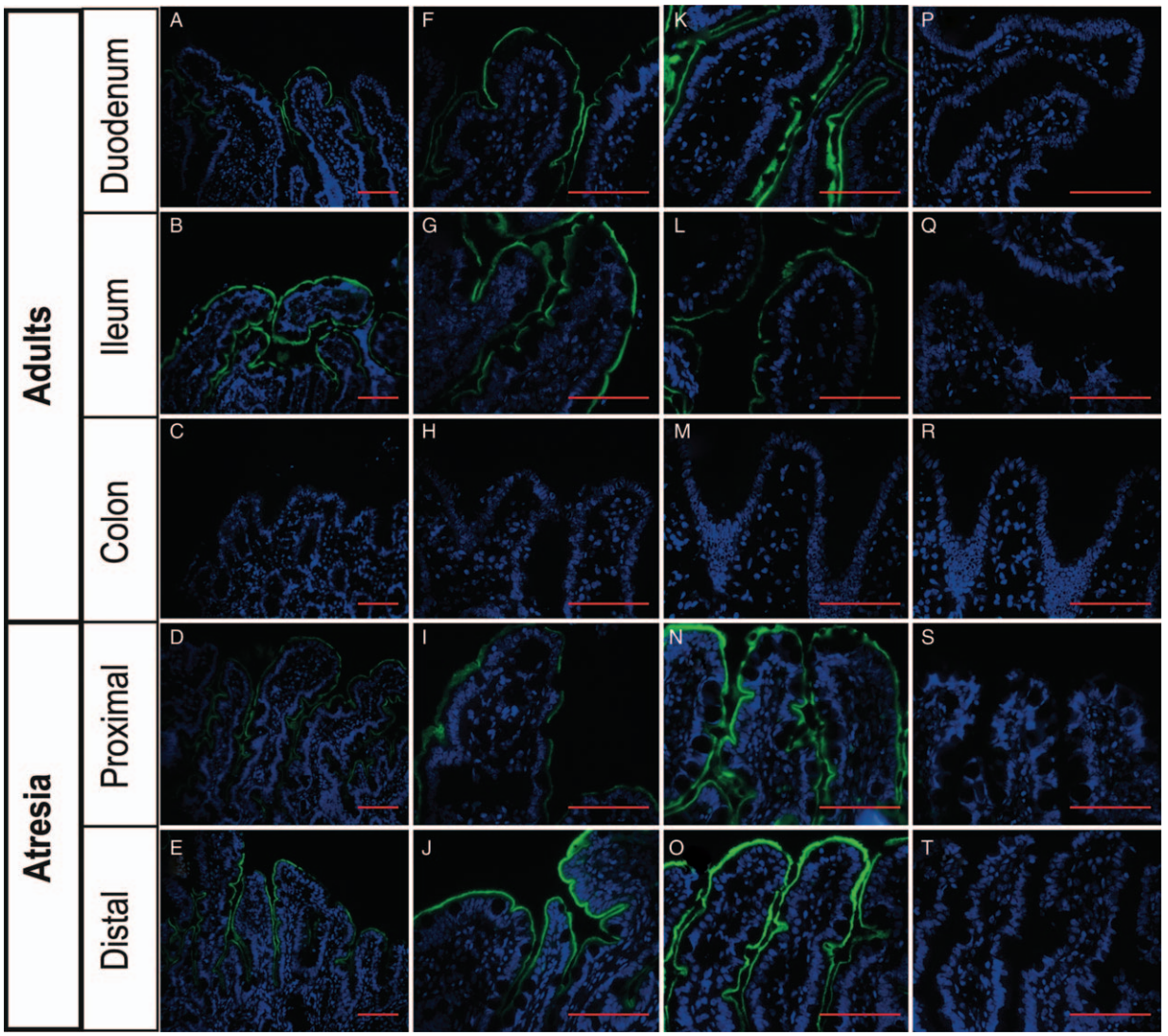

FIGURE 4. Immunofluorescence staining of SGLT1 (green). Cellular DNA (A–T; DAPI; blue) is shown to display the nuclei. Tissue specimens are derived from adult duodenum (A, F, and K), terminal ileum (B, G, and L), and ascending colon (C, H, and M) as well as from newborns proximal (D, I, and N) and distal (E, J, and O) to JIA. Negative controls without a primary antibody against SGLT1 are shown from adults in duodenum (P), terminal ileum (Q), and ascending colon (R), as well as from newborns proximal (S) and distal to atresia (T). Scale bars = 100 μm. JIA = jejunoileal atresia.

with small intestine (duodenum and ileum). This data, reflecting results previously observed in mice (2) and in humans (*https://www.proteinatlas.org*), might indicate a role of GLUT1 to provide energy to colonocytes, instead of it being part of the intestinal nutrient absorption machinery. Under normal conditions, most of the glucose is absorbed in the small intestine with only limited amounts reaching the colon (2,45,46). Correspondingly, the transporters involved in transcellular glucose absorption are not expressed in colon, in particular, also not the basolateral uniporter GLUT2 that can function as both glucose efflux and uptake pathway. Thus, the higher GLUT1 expression in the colon might be reflective of the need for another basolateral glucose transporter in this part of the GI to mediate the glucose uptake from the bloodstream as an energy source for colonic enterocytes (2).

GLUT7 was shown to be expressed in rodents' small intestine and suggested to localize to the apical membrane (47). Initially described as a fructose and glucose transporter (47), it was recently characterized as an orphan transporter (8). Our data suggest that as for adults, GLUT7 is expressed at the RNA level in human newborns and its expression was not modified by the presence of JIA. Unfortunately, because of the lack of specific antibodies, we could not analyze the localization of GLUT1 or GLUT7 in the human intestine.

Taken together, this study shows that in the newborn human gut, the fructose transporter GLUT5 is not expressed at the protein level, whereas its mRNA is expressed as in adults. In contrast, the mRNAs of transporters GLUT1, GLUT2, GLUT7, and SGLT1 and also the transport proteins GLUT2 and SGLT1 are shown to be expressed at comparable levels in newborn and adult small intestine. In addition, gene and protein expression of the assessed monosaccharide transporters was shown not to be affected by in utero small intestinal obstruction (JIA). Finally, with the exception of GLUT1 that is the highest expressed in colon, all other monosaccharide transporters assessed in this study are involved in the

absorption of luminal carbohydrates and display decreasing expression levels from proximal to distal along the intestine.

It remains unclear at what age GLUT5 protein is expressed in human enterocytes. It would hence be valuable to assess intestinal GLUT5 expression at different development stages, as well as whether its expression may be stimulated by diet containing fructose. Moreover, a comparison of the transcriptomes proximal versus distal to small intestinal atresia would be of interest to assess more broadly the effect of temporary amniotic fluid exclusion on intestinal gene expression.

REFERENCES

1. Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* 2003;89:3–9.
2. Yoshikawa T, Inoue R, Matsumoto M, et al. Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract. *Histochem Cell Biol* 2011;135:183–94.
3. Ferraris RP, Diamond J. Regulation of intestinal sugar transport. *Physiol Rev* 1997;77:257–302.
4. Ferraris RP. Dietary and developmental regulation of intestinal sugar transport. *Biochem J* 2001;360 (Pt 2):265–76.
5. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. *Physiol Rev* 2011;91:733–94.
6. Wright EM. Glucose galactose malabsorption. *Am J Physiol* 1998;275 (5 Pt 1):G879–82.
7. Douard V, Ferraris RP. Regulation of the fructose transporter GLUT5 in health and disease. *Am J Physiol Endocrinol Metab* 2008;295:E227–37.
8. Ebert K, Ludwig M, Geillinger KE, et al. Reassessment of GLUT7 and GLUT9 as putative fructose and glucose transporters. *J Membr Biol* 2017;250:171–82.
9. Yu M, Yongzhi H, Chen S, et al. The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis. *Oncotarget* 2017;8:43356–67.
10. Mobassaleh M, Montgomery RK, Biller JA, et al. Development of carbohydrate absorption in the fetus and neonate. *Pediatrics* 1985;75 (1 Pt 2):160–6.
11. Jiang L, David ES, Espina N, et al. GLUT-5 expression in neonatal rats: crypt-villus location and age-dependent regulation. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G666–74.
12. Suzuki T, Douard V, Mochizuki K, et al. Diet-induced epigenetic regulation in vivo of the intestinal fructose transporter Glut5 during development of rat small intestine. *Biochem J* 2011;435:43–53.
13. Drozdowski LA, Clandinin T, Thomson AB. Ontogeny, growth and development of the small intestine: understanding pediatric gastroenterology. *World J Gastroenterol* 2010;16:787–99.
14. Dyer J, Wood IS, Palejwala A, et al. Expression of monosaccharide transporters in intestine of diabetic humans. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G241–8.
15. Hong CR, Han SM, Jaksic T. Surgical considerations for neonates with necrotizing enterocolitis. *Semin Fetal Neonatal Med* 2018;23:420–5.
16. Weiss PA, Hofmann H, Winter R, et al. Amniotic fluid glucose values in normal and abnormal pregnancies. *Obstet Gynecol* 1985;65:333–9.
17. Gurekian CN, Koski KG. Amniotic fluid amino acid concentrations are modified by maternal dietary glucose, gestational age, and fetal growth in rats. *J Nutr* 2005;135:2219–24.
18. Louw JH, Barnard CN. Congenital intestinal atresia; observations on its origin. *Lancet* 1955;269:1065–7.
19. Trahair JF, Harding R, Bocking AD, et al. The role of ingestion in the development of the small intestine in fetal sheep. *Q J Exp Physiol* 1986;71:99–104.
20. Cellini C, Xu J, Buchmiller TL. Effect of esophageal ligation on small intestinal development in normal and growth-retarded fetal rabbits. *J Pediatr Gastroenterol Nutr* 2006;43:291–8.
21. Cohen IT, Greecher CP. Nutritional status following surgical correction of congenital gastrointestinal anomalies. *J Pediatr Surg* 1979;14:386–9.
22. Vuille-dit-Bille RN, Camargo SM, Emmenegger L, et al. Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids* 2015;47:693–705.
23. Davidson NO, Hausman AM, Ifkovits CA, et al. Human intestinal glucose transporter expression and localization of GLUT5. *Am J Physiol* 1992;262 (3 Pt 1):C795–800.
24. Goran MI, Martin AA, Alderete TL, et al. Fructose in breast milk is positively associated with infant body composition at 6 months of age. *Nutrients* 2017;9:pii: E146.
25. Douard V, Ferraris RP. The role of fructose transporters in diseases linked to excessive fructose intake. *J Physiol* 2013;591:401–14.
26. Ebert K, Witt H. Fructose malabsorption. *Mol Cell Pediatr* 2016;3:10.
27. Patricolo M, Noia G, Rossi L, et al. An experimental animal model of intestinal obstruction to simulate in utero therapy for jejunoileal atresia. *Fetal Diagn Ther* 1998;13:298–301.
28. Meier CF, Camargo SMR, Hunziker S, et al. Intestinal IMINO transporter SIT1 is not expressed in human newborns. *Am J Physiol Gastrointest Liver Physiol* 2018;315:G887–95.
29. Buchmiller TL, Shaw KS, Chopourian HL, et al. Effect of transamniotic administration of epidermal growth factor on fetal rabbit small intestinal nutrient transport and disaccharidase development. *J Pediatr Surg* 1993;28:1239–44.
30. Dasgupta S, Arya S, Choudhary S, et al. Amniotic fluid: Source of trophic factors for the developing intestine. *World J Gastrointest Pathophysiol* 2016;7:38–47.
31. Sangild PT, Schmidt M, Elnif J, et al. Prenatal development of gastrointestinal function in the pig and the effects of fetal esophageal obstruction. *Pediatr Res* 2002;52:416–24.
32. Schaart MW, Yamanouchi T, van Nispen DJ, et al. Does small intestinal atresia affect epithelial protein expression in human newborns? *J Pediatr Gastroenterol Nutr* 2006;43:576–83.
33. Camargo SM, Vuille-dit-Bille RN, Mariotta L, et al. The molecular mechanism of intestinal levodopa absorption and its possible implications for the treatment of Parkinson's disease. *J Pharmacol Exp Ther* 2014;351:114–23.
34. Balen D, Ljubojevic M, Breljak D, et al. Revised immunolocalization of the Na+-D-glucose cotransporter SGLT1 in rat organs with an improved antibody. *Am J Physiol Cell Physiol* 2008;295:C475–89.
35. Madunic IV, Breljak D, Karaica D, et al. Expression profiling and immunolocalization of Na(+)-D-glucose-cotransporter 1 in mice employing knockout mice as specificity control indicate novel locations and differences between mice and rats. *Pflugers Arch* 2017;469:1545–65.
36. Wright EM, Loo DD, Hirayama BA, et al. Surprising versatility of Na+-glucose cotransporters: SLC5. *Physiology (Bethesda)* 2004;19:370–6.
37. Verrey F, Singer D, Ramadan T, et al. Kidney amino acid transport. *Pflugers Arch* 2009;458:53–60.
38. Chen J, Williams S, Ho S, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. *Diabetes Ther* 2010;1:57–92.
39. Medina RA. Owen GI Glucose transporters: expression, regulation and cancer. *Biol Res* 2002;35:9–26.
40. Takata K, Hirano H. Kasahara M transport of glucose across the blood-tissue barriers. *Int Rev Cytol* 1997;172:1–53.
41. Ruderisch N, Virgintino D, Makrides V, et al. Differential axial localization along the mouse brain vascular tree of luminal sodium-dependent glutamine transporters Snat1 and Snat3. *J Cereb Blood Flow Metab* 2011;31:1637–47.
42. Carvalho KC, Cunha IW, Rocha RM, et al. GLUT1 expression in malignant tumors and its use as an immunodiagnostic marker. *Clinics (Sao Paulo)* 2011;66:965–72.
43. Haber RS, Rathan A, Weiser KR, et al. GLUT1 glucose transporter expression in colorectal carcinoma: a marker for poor prognosis. *Cancer* 1998;83:34–40.
44. Ito T, Noguchi Y, Udaka N, et al. Glucose transporter expression in developing fetal lungs and lung neoplasms. *Histol Histopathol* 1999;14:895–904.
45. Martin AM, Lumsden AL, Young RL, et al. The nutrient-sensing repertoires of mouse enterochromaffin cells differ between duodenum and colon. *Neurogastroenterol Motil* 2017;29:doi: 10.1111/nmo.13046.
46. Ferraris RP, Yasharpour S, Lloyd KC, et al. Luminal glucose concentrations in the gut under normal conditions. *Am J Physiol* 1990;259 (5 Pt 1):G822–37.
47. Li Q, Manolescu A, Ritzel M, et al. Cloning and functional characterization of the human GLUT7 isoform SLC2A7 from the small intestine. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G236–42.